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Synthesis, characterization and biocompatibility of poly(2-ethyl-2-oxazoline)–poly(D,L-lactide)–poly(2-ethyl-2-oxazoline) hydrogels

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ABSTRACT

A novel thermoreversible hydrogel based on poly(2-ethyl-2-oxazoline)-derived amphiphilic triblock copolymer, poly(2-ethyl-2-oxazoline)–poly(D,L-lactide)–poly(2-ethyl-2-oxazoline) (PEOz–PLA–PEOz), was developed. The synthesis of PEOz–PLA–PEOz was carried out by coupling monohydroxylated PEOz–PLA diblocks with adipoyl chloride as coupling agent and dimethylamino pyridine as catalyst. The tube inverting and rheological tests showed that triblock copolymers had sol–gel–sol transition behavior with increasing temperature, and the gelation was found to be thermoreversible. The critical gelation concentration, the sol–gel transition temperature at a given concentration depended on the EOz/LA ratio and the molecular weight of PEOz. Scanning electron microscopy observation revealed that the resultant bulky gel exhibited an interconnected porous three-dimensional (3D) microstructure after freeze-drying. In addition, the hydrogels showed good cytocompatibility *in vitro*. MTT assays revealed that the human skin fibroblast cells encapsulated within the hydrogels were viable and proliferated inside the 3D scaffold. This newly described thermoreversible hydrogel demonstrated attractive properties to serve as cell matrix for a variety of tissue engineering applications or pharmaceutical delivery vehicles.

1. Introduction

In situ thermosensitive hydrogels have received much more attention in recent years in the field of tissue engineering and controlled drug delivery. The aqueous solution of thermosensitive polymers is a free-flowing sol at room temperature, but becomes a gel when the temperature increases to body temperature. Since the polymers exhibited a sol–gel transition with increasing temperature, it has been suggested as a promising injectable system, which may provide a convenient means for injectable drug delivery [1–4]. Also, owing to biocompatibility, the in situ gelation is also beneficial for cell encapsulation and for providing a scaffold for tissue regeneration [5–8]. Among the different thermosensitive polymers, triblock copolymers consisting of balanced hydrophobic and hydrophilic blocks have been some of the most intensively investigated thermosensitive biomaterials in the past few decades [9]. Hydrogels of triblock copolymers such as poly(ethylene oxide)(PEO)–poly(propylene oxide)(PPO)–poly(ethylene oxide)(PEO), know as Pluronic or Poloxamer, have been studied extensively [8,10]. However, the major drawback for the use of these copolymers in the fields of tissue engineering and drug delivery is that they are non-biodegradable and could be accumulated in the body [11,12]. The importance of biodegradability for a biomaterial is self-evident, since it prevents the risk of a chronic foreign body reaction, which usually occurs with the permanent presence of non-biodegradable materials. Thus, the application of PEO–PPO–PEO copolymers in biomedical fields has been greatly restricted. The biodegradable triblock copolymers composed of poly(ethylene glycol) (PEG) as hydrophilic block and polyester as hydrophobic block, such as poly(L-lactic acid) (PLLA), poly(D,L-lactic acid) (PDLA), poly(lactic acid-*co*-glycolic acid) (PLGA) and poly-(D,L-lactic acid-*co*-ε-caprolactone)(PDLA-*co*-PCL), have therefore received special attention in recent years [4,13–18]. In addition, these copolymers exhibited an evident decrease in the critical micelle concentration (CMC) in comparison with PEO–PPO–PEO copolymers [19,20]. In previous publications, the majority of triblock copolymers that have so far been investigated for use in drug delivery and tissue engineering applications have focused on those with the hydrophilic block of PEG, owing to its biocompatible, non-toxic, non-antigenic and non-immunogenic properties.

Poly(2-ethyl-2-oxazoline) (PEOz), a water-soluble polyelectrolyte which has as low toxicity and higher hydrophilicity than that of PEG [21], has been approved by the US Food and Drug

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Administration (FDA) for use as an indirect food additive. Liposomes on which PEOz was grafted have been shown to circulate for a long time in the blood and to be associated with a reduction of the clearance rates in spleen and liver [21]. Polymer complexes composed of PEOz and poly(methacrylic acid) have been designed for the on–off insulin-released matrixes [22]. Only very recently, PEOz was used for the first time to design temperature-sensitive polymer materials with reasonable potential for use in biomaterials and specific drug delivery [23]. Wang et al. successfully synthesized a series of PLLA–PEOz–PLLA triblock copolymers, which were both thermal- and pH-sensitive [24]. PEOz, despite its low toxicity and superb chemical versatility, has been considerably less studied than PEG, and only a few biomedical applications of this polymer have been contemplated. PLA is a well-known FDA-approved non-toxic, biodegradable and biocompatible material, which has been widely used in the biomedical field. Owing to the integration of the respective advantages of PEOz and PLA, PEOz–PLA–PEOz copolymer might have even wider applications in the biomedical field.

Given the above benefits of using PEOz for biomaterial design and the fact that none of PEOz-based triblock copolymers with dual hydrophilic block has been developed for in situ drug and cell delivery to the best of the present authors' knowledge so far, a new series of amphiphilic triblock copolymers based on hydrophilic PEOz and hydrophobic PLA were synthesized and characterized in the present study. The unique properties of the resulting PEOz–PLA–PEOz triblock copolymers with respect to thermal behavior, rheological property and micellization behavior were also examined. The temperature-dependent phase transition behavior of these copolymers in aqueous medium was investigated in detail. Finally, the biocompatibility of these copolymers was evaluated with respect to their cytotoxicity and cell proliferation. By examining hydrogel formation, cell encapsulation and cell viability, the possibility of using the PEOz–PLA–PEOz hydrogel as a general long-term living cell-based tool was explored, and the hydrogel's potential for use in cell-based applications was demonstrated. The information obtained from this study is important and meaningful in that it helps to elucidate the inherent properties of the PEOz–PLA–PEOz hydrogel, and explores its possible applications.

2. Materials and methods

2.1. Materials

Before use, 2-ethyl-2-oxazoline (Aldrich) was purified by vacuum distillation over calcium hydride. Acetonitrile and dichloromethane purchased from Sinopharm Medicine Holding Co., Ltd. (Shanghai, China) were dried over calcium hydride and distilled under nitrogen. Methyl *p*-toluenesulfonate (MeOTs, Aldrich) and adipoyl chloridewas purchased from Acros were distilled before use. D,L-Lactide obtained from Daigang Biological Technology Co., Ltd. (Shandong, China) was purified by recrystallization from ethyl acetate before use. Stannous octoate was purchased from Sigma (St Louis, MO, USA). Dimethylamino pyridine (DMAP) from Acros Organics (Geel, Belgium) was used as received. Pluronic F127 was supplied by BASF (Mount Olive, NJ, USA). Tetrazolium salt MTT, trypsin and sulforhodamine B (SRB) sodium salt (SRB) were all purchased from Sigma–Aldrich (St Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and trypsin–EDTA were all obtained from M&C Gene Technology (Beijing, China).

The 25 cm^2 and 75 cm^2 plastic culture flasks and the 12-well and 96-well tissue culture plate were obtained from Costar (Corning Incorporated, USA).

2.2. Synthesis and purification of the PEOz–PLA–PEOz triblock copolymers

PEOz–PLA–PEOz was prepared using a three-step reaction procedure. First, monohydroxyl poly(2-ethyl-2-oxazoline) (PEOz-OH) with different molecular weights was synthesized by the cationic ring-opening polymerization of 2-ethyl-2-oxazoline (EOz) using MeOTs as an initiator, as reported previously, with a slight modification (Fig. 1) [25]. Briefly, a solution of EOz (30 g) and MeOTs (6.5 g) in acetonitrile (100 ml) was added to the pre-dried reaction flask, and then stirred at reflux (100 °C) in an oil bath for 24 h under nitrogen. After cooling to room temperature, the resulting product was added to 0.1 M of methanolic KOH and the reaction was maintained for 4 h to introduce hydroxyl groups at the end of the PEOz chain. The crude product was flowed through silica gels and precipitated in diethyl ether, and then the product, PEOz-OH, was vacuum-dried for 24 h. Secondly, the PEOz–PLA diblock copolymers were synthesized from D,L-lactide and PEOz-OH using stannous octoate as the catalyst, as described previously, with little modification [25]. In brief, D,L-lactide (14 g) and PEOz-OH (9.7 g) were dissolved in dry methyl benzene (150 ml) under nitrogen and then stannous octoate (30 mg) was added. The mixture was refluxed at 120 °C for 24 h under a nitrogen atmosphere. After being allowed to cool, the product was isolated by precipitation in diethyl ether and then vacuum-dried for 24 h to obtain white solid powder. Finally, a series of PEOz–PLA–PEOz triblock copolymers

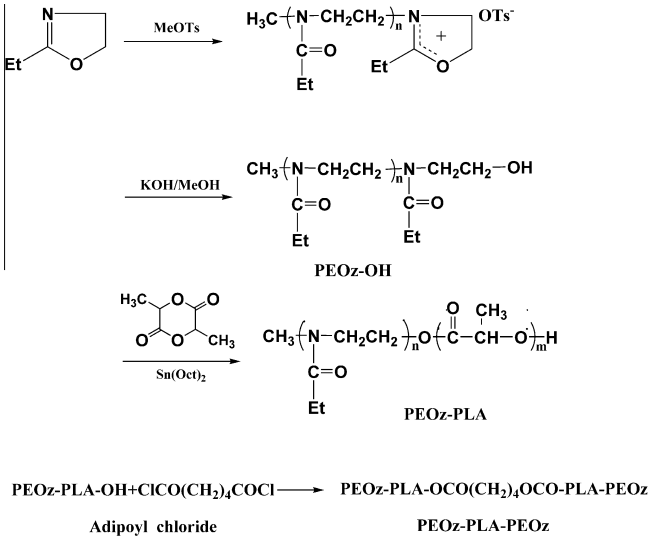

Fig. 1. Synthetic scheme of the PEOz–PLA–PEOz triblock copolymer.

Table 1
Molecular characteristics of PLA/PEOz diblock and triblock copolymers.

Polymer	EOz/LA[a]
EOz_{850}–LA_{810}	0.76
EOz_{850}–LA_{1620}–EOz_{850}	0.76
EOz_{850}–LA_{1160}	0.53
EOz_{850}–LA_{2320}–EOz_{850}	0.53
EOz_{1080}–LA_{1200}	0.62
EOz_{1080}–LA_{2400}–EOz_{1080}	0.62
EOz_{800}–LA_{1500}	0.39
EOz_{800}–LA_{3000}–EOz_{800}	0.39
EOz_{1000}–LA_{1500}	0.49
EOz_{1000}–LA_{3000}–EOz_{1000}	0.49

[a] Estimated by ^1H NMR.